ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5531

91-04721/

Toxins produced by marine bacteria

U. Shimizu

Original title: Kaiyo Saikin to Dokuso

In: Shokuhin Eisei Kenkyu/[Food Hygiene Study] 40(6): 47-58, 1990

Original language: Japanese

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1991

15 typescript pages

Secretary of State / Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU / BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

Translated from - Traduction de	Into - En
Japanese	English

Author - Auteur

Ushio SHIMIZU

Title in English or French – Titre anglais ou français

Toxins produced by Marine Bacteria

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

Kaiyo Saikin to Dokuso

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Shokuhin Eisei Kenkyu

Reference in English or French – Référence en anglais ou français

Food Hygiene Study

Publisher - Editeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
	Year / Année	Volume	Issue No. / Numéro	47 – 58
Place of Publication / Lieu de publication	1990	40	(6)	Number of typed pages / Nombre de pages dactylographiées: 14

Requesting Department / Ministère-Client: DFO

Translation Bureau No. / Notre dossier nº: 3850533

Branch or Division / Direction ou Division: Scientific Pubs / Communications

Translator (Initials) / Traducteur (Initiales): PS

Person requesting / Demandé par: J. Stewart , BIO, DFO
Dartmouth, N.S.

Your Number / Votre dossier nº:

Date of Request / Date de la demande: March 26, 1991

MAY 13 1991

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Canada

SEC 5-111 (84-10)

Secretary of State—Secrétariat d'État

MULTILINGUAL TRANSLATION DIRECTORATE **DIRECTION DE LA TRADUCTION MULTILINGUE**

TRANSLATION BUREAU **BUREAU DE LA TRADUCTION**

Client's No. — N° du client	Department — Ministère	Division/Branch — Division/Direction	City — Ville
	DFO	Scientific Pubs	Ottawa
Bureau No. — N° du Bureau	Language — Langue	Translator — Traducteur	MAY 13 1991
3850533	Japanese	PS	

Toxins produced by Marine Bacteria

(Kaiyo Saikin to Dokuso)

by Ushio SHIMIZU

Ocean Research Institute, University of Tokyo

in Shokuhin Eisei Kenkyu (Food Hygiene Study), Vol. 40 (6), 1990, pp. 47-58

I. Foreword

It is common knowledge that marine animals and plants produce all kinds of physiologically active substances. It is said that at the present time, the chemical structures of up to about 3,000 types of such physiologically active substances are known. It was of course believed that such substances were produced for the vital needs of the very animals and plants possessing these substances.

However, there appeared successive reports to the effect that such physiologically active substances, and more particularly the toxins were actually produced by bacteria. The physiologically active substances mentioned in this respect are toxins such as puffer poison (tetrodotoxin), paralytic shellfish poison, palytoxins, neosurugatoxins, prosurugatoxins as well as antiviral and antileukemic substances. It seems that we will see more and more reports of such kind.

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

II. Puffer poison

1. Distribution of puffer poison in the animal world

Useless to say, tetrodotoxin, the puffer poison is a potent paralytic

p. 48

poison acting on the nerves and muscles. The human lethal dose is estimated to be 0.5-1 mg. It was in 1963 that one discovered that this poison formerly believed to be contained only in fish of the puffer family was also held by other animals. In that year, Mosher et al[1] reported that the toxin called talicatoxin (?), contained in the eggs of the California newt was nothing else than tetrodotoxin. Later through the efforts of foreign and Japanese researchers, and mainly of Hashimoto and Noguchi of Japan, it was demonstrated

p. 49

that this toxin was contained in numerous animals, some of which are marine animals.

Table 1 shows the animals presently known to contain the puffer poison.

2. The origin of the puffer poison

As Table 1 indicates, the puffer poison is found among numerous animals such as fish, molluscs, crustaceans, and Polychaeta, regardless of their phyletic origin.

Furthermore, the poison contents are not constant in the particular animals. There are extremely great differences depending on the individuals or within the same individuals depending on the areas and seasons. Moreover, Matsui et al revealed the astonishing fact that there was no poison at all in cultured puffers [2]. These facts lead to surmise that the puffer poison was not produced by the fish itself, but that it might be ingested by the animal from the outside via its food for example.

On the basis of such estimations, numerous researchers studied the food chain ending with the puffer, and discovered successively puffer poison in animals preyed upon by larger animals. At the end of their research, Yasumoto et al [3], and Noguchi et al [4] discovered in 1985-1986 the bacteria producing the puffer poison.

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

3. The production of puffer poison by marine bacteria

Yasumoto et al isolated from calciphilous grass of Ishigakijima, and Noguchi et al from the intestines of crabs bacteria producing tetrodotoxin. Within an extremely short time after the above discoveries, numerous researchers discovered particular bacteria producing this same toxin in puffer, pongee gobies (Gobius criniger), starfish, hiramushi, nemertines, hyomondako (Octopus maculosus), horseshoe crabs and the like.

Narita et al [5] reported that the bacteria isolated from Togemomijigai (*Astropecten polyacanthus*), a species of starfish produced particularly strong tetrodotoxins, and that these bacteria had been identified as *Vibrio alginolyticus*. It is well known that these are extremely common marine bacteria, and that they are numerous in seawater as well as in the intestines and body surface of marine fish. Of course, the news that such ordinary mixed bacteria produced puffer poison caused wide repercussions in all quarters.

So, together with the Hashimoto group, we examined whether *Vibrio alginolyticus* really produced the tetrodotoxins, and if so, whether the same

p. 51

toxins were produced by the enteritis and other vibrios [6].

First with regard to the strains, we used 18 species, 24 strains chosen among standards obtained from preserving institutions in various countries. After culturing these bacteria in liquid media made for marine bacteria, we gathered the bacterial portion through centrifugation, extracted the toxins from the same with dilute acetic acid, and performed the detection and confirmation of the toxins according to the procedure shown in Fig.1. This is shown in Table 2. We discovered that among the 11 tested species of bacteria of the Vibrio family (*Vibrio, Photobacterium, Aeromonas, Plesiomonas*), up to 7 species including *V. alginolyticus* and the enteritis vibrio produced anhydrotetrodotoxins. In the tests performed at that time, we did not examine *Vibrio cholerae* which is the standard (type) of the Vibrio genus. Later, Tamplin et al of the University of Maryland reported that the *Vibrio cholerae* produced a substance harmful to the sodium ion channel, and Huang et al [7] confirmed that this substance was nothing else than tetrodotoxin.

The *Alteromonas* genus bacteria which along with Vibrio are typical marine bacteria did not produce tetrodotoxin during the tests performed at that time. However, the bacteria producing the puffer poison isolated from calciphilous grasses by Yasumoto et al, and later from the body surface of

puffers were at first believed to be *Pseudomonas*, and later believed to be probably *Alteromonas*. When we examined the phyletic relations of this bacterium, using a 16 S ribosome RNA base sequence, our conclusion was that one of the strains was *Alteromonas*, and the other *Shewanella* [8]. The distinction into the three *Pseudomonas*, *Alteromonas*, *Shewanella* genera is extremely difficult with the traditional methods. By using genetic taxonomy, clear distinctions are possible.

Matsui et al reported that the tetrodotoxin-producing bacteria isolated from the intestines of the Kusa blowfish* were *Shewanella putrefaciens* [9].

p. 52

Besides the bacteria of the Vibrio family, the bacteria of the *Alteromonas* genus and those of the *Shewanella* genera, there are other bacteria producing puffer poison, which as will be explained later, are spread across wide-ranging species. At the present time, there are not that many bacteria which have been identified up to the species level. They are limited to those bacteria listed in Table 3.

4. Tetrodotoxins present in the sea bottom sediments

About 2 years ago, bacteria producing puffer poison were isolated from animals such as puffer, starfish, horseshoe crabs and from plants possessing the poison. This should indicate that many researchers thought that the poison was produced by bacteria attached to those animals and plants or commensal with the same. The fact that there were many bacteria of the Vibrio family among the bacteria producing puffer poison, seemed particularly corroborative of such estimations. It is well known that the intestinal bacteria of marine fish consist almost entirely of vibrios. Some of the latter produce puffer poison, and it is by all means reasonable to believe that they are absorbed by the host fish.

On the other hand however, the fact that cultured puffers contain no poison whatsoever has been revealed by Matsui et al. Yet, according to the studies of Sugita et al, there does not seem to be a clear difference between the bacterial florae found in the cultured puffers and of those found in the free-swimming ones containing the poison [10]. According to Sugita et al,

p. 53

---

* translator's note : *Fugu niphobles* (Jordan & Snyder)

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Vibrio alginolyticus which is believed to produce tetrodotoxin actively is present in about the same quantities in the free-swimming and in the cultured puffers (10^4 - 10^6 / g). Such results contradict the estimation that the poison produced by the intestinal bacteria are at the origin of the poison contained in puffers.

Kigurashi * et al examined the sea bottom sediments as possible origin of puffer poison. They collected sea bottom sediments at depth of 4,000 m in the outer sea, east of The Tokyo Bay and off the Izu archipelago, and made quantitative determinations of the toxins contained (substances harmful to the sodium channel) [11]. As a result, and as shown in Table 4, fairly high concentrations of toxins were detected in the sea bottom sediments. The HPLC analysis of these toxins show that at least the greatest part consists of tetrodotoxin. Quite surprisingly, the amounts of toxins found in sea bottom debris were hardly different in the eutrophicated areas such as the Tokyo Bay on the one hand, and at 4,000 m deep sites of the high sea on the other. Later investigations revealed that even sediments laying 7,000 m deep in the Mariana sea trench contained several + ng/g of toxins.

Miyako et al tested whether bacteria isolated from sea bottom sediments at depths of 4,000 m had the power to produce tetrodotoxins. The results are shown in Table 5. The table shows that a fair portion of the bacteria present

p. 54

in sea bottom sediment produce substances harmful to the sodium channel. In the scope of our investigations, the substances formed were tetrodotoxins.

It is noteworthy that among the bacteria producing puffer poison, there are not only typical marine bacteria such as the aforementioned *Vibrio Alteronomas* but other species of bacteria as well such as *Bacillus*, *Micrococcus* which are mostly observed on land. Miyako et al confirmed that typical terrestrial bacteria such as the actinomycetes produced tetrodotoxins actively. The later investigations and tests conducted by these researchers showed that many of the bacteria isolated from the mud of the Suwa lake in the province of Nagano or from bottom mud at the Inogashira Park in Tokyo produced also puffer poison. These facts indicate that the bacteria producing puffer poison are very widely distributed in the natural world, or suggest that most bacteria might have the capability of producing variable amounts of the same.

---

* Translator's note : reading probable only; other readings possible

5. Accumulation route leading to the puffer poison animals

The results of such studies lead us to the following estimations with regard to the accumulation route of puffer poison inside the bodies of animals, starting with the puffer.

p. 55

Puffer poison is first produced by bacteria in the sea bottom sediments. The produced toxins are thereafter ingested by a series of small and large animals called detritus eaters. Detritus is unstable scrap made from the decomposed bodies of sea animals, plants and excrements. Detritus eaters such as lugworms, shellfish, crabs, sea cucumbers and the like do in fact eat the bacteria attached to detritus. The puffer poison ingested inside the animals enters thereafter the so-called detritus food chain. As the chain progresses stage by stage, the poison is ingested by larger animals and concentrated (Fig. 2).

To ascertain this view, numerous tasks remain to be accomplished.

Even though we know that the puffer poison is concentrated via the detritus food chain, we have no detailed picture of the route. Of course, not all animals involved in this food chain do accumulate the puffer poison. Only a series of animals limited in the number of their species has an especially high resistance to puffer poison, and store it in their bodies. Therefore, one must first know which among the species of animals living on the sea bottom or close to the same, are the species that accumulate puffer poison, and under which conditions they do so.

The criticism that the quantity of puffer poison produced in the culture base by the puffer poison-producing bacteria under in-vitro culture conditions is much smaller than the quantity of blowfish poison held by animals, was formulated by numerous researchers. Regarding this point, one must examine the culture conditions adopted for an active production of puffer poison by the bacteria. Since the quantitative determination of puffer poison is not easy, research in this field is unfortunately still lagging, even though the necessity of such studies is being pointed out.

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

III. Paralytic shellfish poison

One may say that in Japan, paralytic shellfish poison is generally much less well known than blowfish poison. In foreign countries however, the opposite is true. Much more importance is given to paralytic shellfish poison, so that there is much more research in this field.

With regard to this poison, it had already been established in the twenties that it was produced by dinoflagellate chaetophoraceae of the *Alexandrium* genus (thought at some earlier time to belong to the *Protogoniaulax* genus), and that poisoning occurred as shellfish such as scallops, hard-shell mussels ate these algae.

However, Kodama et al expressed in 1984 the opinion that the poison was not produced by this phytoplankton, but by bacteria buried in the cell as commensals of the nucleus. This announcement caused wide repercussions in

p. 56

Japan and abroad. At present, there are numerous critics of the symbiotic theory. Yet, Kodama et al have isolated successively bacteria producing paralytic shellfish poison from plankton cultures or from seawater [12].

More recently, Yasumoto et al, Noguchi et. and ourselves have found bacteria producing paralytic shellfish poison, and it seems practically certain that the bacteria producing this toxin are rather widely distributed.

Meanwhile, tests are being conducted to see whether the above *Alexandrium* phytoplankton produces paralytic shellfish poison in sterilized cultures. Numerous researchers believe that even if one supposes that bacteria produce the toxin, the most likely main agent of shellfish poisoning could indeed be phytoplankton.

To elucidate this problem too, it is like in the case of the above puffer poison, absolutely necessary to know under what conditions and in what quantities the toxin is produced by the bacteria.

IV. The other toxins

1. Polytoxin

The palytoxin was isolated from *Palythoa toxica*, a species of soft coral, and purified for the first time in the eighties by Moore et al of the

University of Hawai. The toxicity to mouse (450 ng/kg) is about 20 times that of the puffer poison. As the volumes of this toxin contained in *Palythoa* differ widely according to individuals and regions, Moore et al thought that maybe the organism producing this toxin was some bacterium parasitizing the soft coral with which it lived, and after much exertion, separated one strain of bacterium of the *Vibrio* genus thought to produce the toxin. However, during preservation, this bacterium strain lost completely its capacity of producing the toxin [13].

2. Surugatoxin, neosurugatoxin

These toxins were extracted and purified by Kosuga et al as a substance causative of food poisoning, originated from ivory shells produced in Ganyudo, Shizuoka Prefecture. The structure of this substance was determined. Kosuga et al reported that bacteria isolated from the poisonous ivory shells produced this toxin [14]. The bacterium was a coryneform. The distribution of this bacterium, the conditions for its productivity and the like are not yet known.

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Tables and Graphs

表1 フグ毒をもつ動物 Table 1

動植物の種類 A		学名 B
脊椎動物		
a 両生類	イモリ aa	*Taricha torosa*
		T. rivularis
		T. granulosa
		Notophthalmus viridescens
		その他
	カエル ab	*Atelopus varius*
		A. chiriquiensis
b 魚類	フグ科魚類 ba	*Fugu* spp.
	ツムギハゼ bb	*Gobius criniger*
	アオブダイ bc	*Ypsiscarus ovifrons*
c 棘皮動物	トゲモミジガイ ca	*Astropecten polyacanthus*
	ヒラモミジガイ cb	*A. latespinosus*
	モミジガイ cc	*A. scoparius*
d 毛顎動物	ヤムシ da	*Parasagitta elegans*
		Flaccisagitta scrippsae
節足動物		ea
e 甲殻類	スベスベマンジュウガニ	*Atergatis floridus*
	ウモレオウギガニ eb	*Zosimus aeneus*
f 剣尾類	カブトガニ fa	*Carcinoscorpius rotundicauda*
g 軟体動物		
腹足類	ボウシュウボラ ga	*Charonia sauliae*
	gb オオナルトボラ	*Tutufa lissostoma*
	バイ gc	*Babylonia japonica*
	gd ハナムシロガイ	*Zeuxis siquijorensis*
	アラレガイ ge	*Niotha clathrata*
	gf カコボラ	*Cumatium echo*
	テングニシ gg	*Pugilina ternotana*
h 頭足類	ヒョウモンダコ	*Octopus maculosus*
i 扁形動物	オオツノヒラムシ ia	*Planocera multitentaculata*
	ツノヒラムシ ib	*P. reticulata*
j 紐形動物	ミドリヒモムシ ja	*Lineus fuscoviridis*
	クリザヒモムシ jb	*Tubulanus punctatus*
	ニロコムシ jc	*Lepidonotus* sp.
k 環形動物	エラコ	*Pseudopotamilla occelata*
l 海藻類		
紅藻類	石灰藻	*Jania* sp.

Table 1 Animals holding puffer poison

A. animal species, B. Scientific name
a. vertebrates, amphibians, aa. newt, ab. and others, ac. frog
b. fish, ba. fish of the puffer family, bb. pongee goby, bc. "blue parrot fish" (a species of sea bram)
c. echinoderms, ca. togemomijigai (a type of starfish), cb. hiramomijigai (a type of starfish), cc. momijigai (a type of starfish)
d. Chaetognatha, da. arrowworm
e. arthropods, crustaceans, ea. subesubemanjugani (a crab), eb. umoreuogani (a crab)

f. Xiphosura, fa. horseshoe crab

g. molluscs, gastropods, ga. boshubora (a shellfish) gb. onarutobora (a shellfish), gc. ivory shell, gd. hanamushirogai (a shellfish), ge. araregai (a shellfish), gf. kakobora (a shellfish), gg. tengunishi (a shellfish)

h. cephalopods, hyomondako (an octopus)

i. Platyhelminthes (flat worms), ia. otsunohiramushi (a polyclad), ib. tsunohiramushi (a polyclad)

j. Nemerteans, ja. midorihimomushi (green) , jb. kurigehimomushi (an anopla), jc. urokomushi (seems to have have scales)

k. Annelids, erako (a sedentary polychaete)

l. Marine algae, red algae, calciphilous algae

UNEDITED TRANSLATION
For reference only
TRANSLATION NON REVISEE
Information assistance

Fig. 1 Analysis of the puffer poison, tetrodotoxin linking substances

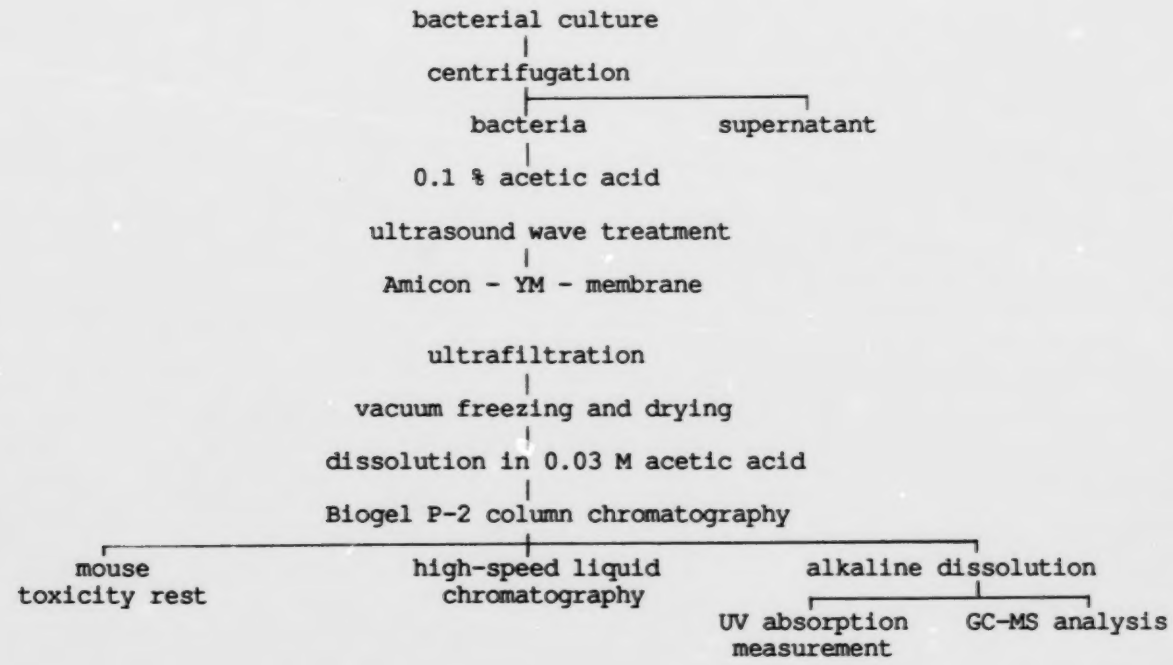

Table 2 Anhydrotetrodotoxin production by standard strains

a. tested strain, b. number of strains, c. number of anh. TTX-producing strains

Table 3. The bacteria producing puffer poison

a. scientific name, b. according to the report of Matsui et al (1987)

Table 4 Tetrodotoxin concentrations in sea bottom sedimental matters and the bacterial count

a. site, aa. Tokyo Bay, ab. waters east off the Izu archipelago

b. depth, c. sedimental stratum, d. tetrodotoxins, e. bacterial count

Table 5 Puffer poison-producing bacteria in the sea bottom sediments at the depth of 4,000 m east off the Izu archipelago

a. generic composition of the strains, b. test bacterial counts, c. number of strains producing substances interfering with the sodium ion channel

Fig. 2 Concentration of the puffer poison through the food chain

Table 2 表2 標準菌株による無水型テトロドトキシンの産生

試験菌株	菌株数	anh.TTX 産生菌株数
Vibrio alginolyticus	2	2
V. anguillarum	2	2
V. costicolus	1	1
V. fischeri	1	0
V. marinus	1	0
V. parahaemolyticus	2	2
Photobacterium fischeri	1	0
P. phosphoreum	1	1
Aeromonas hydrophila	1	0
A. salmonicida	1	1
A. harveyi	1	0
Plesiomonas shigelloides	1	1
Escherichia coli	1	0
Alteromonas communis	1	0
A. haloplanktis	1	0
A. nigrifaciens	1	0
A. undina	1	0
A. vaga	1	0

Table 3

表3 フグ毒を産生する細菌

学名
Vibrio alginolyticus
V. anguilallum
V. cholerae
V. costicola
V. parahaemolyticus
Listonella pelagia
*Shewanella putrifaciens**
Shewanella alga sp. nov.
Photobacterium phosphoreum
Aeomonas hydrophyla
Plesiomonas shigelloides
Alteromonas tetrodonis sp. nov.

(*は松居らの報告 (1987))

Table 4　表4　海底堆積物中のテトロドトキシンの濃度と細菌数

場所	深さ (m)	堆積層 (cm)	テトロドトキシン (ng/g)	細菌数 (No/g)
東京湾 A	21	0～ 3	57	1.8×10^{3}
		3～ 6	38	1.3×10^{3}
		6～11	37	−
		11～17	41	−
東京湾 B	82	0～ 3	30	1.8×10^{3}
		3～ 6	27	8.5×10^{4}
		11～17	54	1.0×10^{4}
伊豆諸島東方沖 C	4033	0～ 2	90	3.3×10^{3}
		2～ 5	40	1.1×10^{4}
		5～ 8	65	5.5×10^{3}
		8～11	37	7.0×10
		15～18	25	5.5×10

Table 5

表5　伊豆諸島東方の4,000m の深海の海底堆積中のフグ毒産生細菌

菌株の属組成	試験菌株数	ナトリウムイオンチャネル阻害物質産生菌株数
Bacillus	10	5
Micrococcus	7	4
Aeromonas	1	1
Vibrio	1	1
Alteromonas	9	5
Acinetobacter	6	3
Moraxella	3	2
Pseudomonas	2	0
Alcaligenes	4	0
Flavobacterium	1	0
Not identified	5	1
Total	49	22

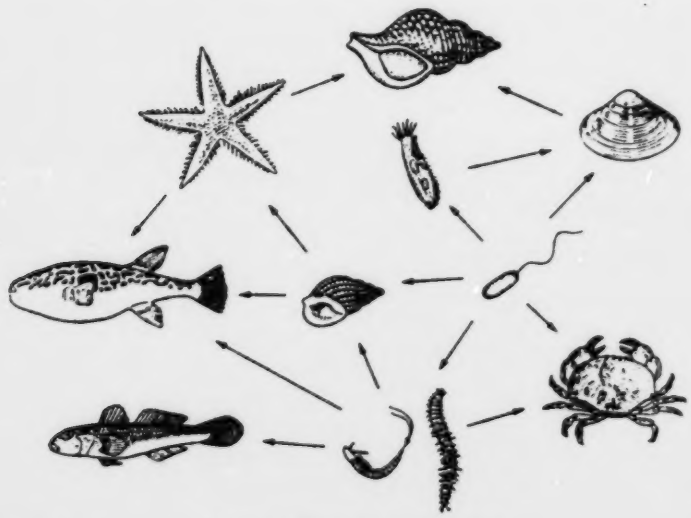

Fig. 2　図2　食物連鎖によるフグ毒の濃縮

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Bibliography

7. Huang, D.F., Tamao NOGUCHI, Ushio SHIMIZU, Kanehisa HASHIMOTO : *Vibrio cholerae* no tetorodotokishin sanseino (The capacity of producing *Vibrio cholerae* tetrodotoxins), Showa 62-nendo Nippon Suisan Gakkai Shuki Taikai Koen Yoshi (Summaries of the lectures given at the 1987 fall convention of the Japanese Society for Scientific Fisheries), p. 189 (1987)

9. Takashi MATSUI, Kentaro KODAMA, Akira ISHII, Kunio YAMAMORI, Chiaki SHIMIZU : Kusafugu Chonai Saikin ni yoru Tetrodotoxin no Sansei (The production of tetrodotoxins by the intestinal bacteria of the Kusa blowfish), same source and page as in 7. above

1. Mosher, H.S., Fuhrman, F.A., Buchwald, H.D., and Fisher, H.G., Tarichatoxin-tetrodotoxin: a potent neurotoxin. Science., 144, 1100-1110

(1964)

2) Matsui, T., Sato, H., Hamada, S., and Shimizu, C.: Comparison of toxicity of the cultured and wild puffer fish *Fugu niphbles*. Nippon Suisan Gakkaishi, 48, 253 (1982)

3) Yasumoto, T., Yasumura, D., Yotsu, M., Michishita, T., Endo A., and Kotaki, Y.: Bacterial production of tetrodotoxin and anhydrotetrodotoxin. agric., Biol., Chem., 50: 793-795(1986)

4) Noguchi, T., J.-K. Jeon, Arakawa, O., Sugita, H., Deguchi, Y., Shida, Y., and Hashimoto, K.: Occurrence of tetrodotoxin and anhydrotetrodotoxin in *Vibrio* sp. isolated from the intestines of a xanthid crab, *Atergatis floridus*. J. Biochem., 99, 311-314 (1986)

5) Narita, H., Matsubara, S., Miwa, N., Akahane, S., Murakami, M., Goto, T., Nara, M., Noguchi, T., Shida, T., and Hashimoto, K.,: *Vibrio alginolyticus*, a TTX-producing bacterium isolated from the starfish *Astropecten polyacanthus*. Nippon Suisan Gakkaishi, 53, 617-621 (1987)

6) Simidu, U., Noguchi, T., Huang, D.-F., Shida, Y., and Hashimoto, K.: Marine bacteria which produce tetrodotoxin. Appl. Environ. Microbiol., 53, 1714-1715 (1987)

7) 黄登福，野口玉雄，清水潤，橋本周久： *Vibrio cholerae* のテトロドトキシン産生能，昭和62年度日本水産学会秋期大会講演要旨，p. 189 (1987)

8) Simidu, U., Kita-Tsukamoto, K., Yasumoto, T., and Yotsu, M.: Taxonomy of four marine bacterial strains that produce tetrodotoxin. Int. J. Syst. Bacteriol., 投稿中 (1990)

9) 松居隆，小玉健太郎，石井晃，山森邦夫，清水千秋：クサフグ腸内細菌によるテトロドトキシンの産生，昭和62年度日本水産学会秋期大会講演要旨，p. 189 (1987)

10) Sugita, H., Noguchi, T., Furuta, M., Harada, T., Murata, O., Hashimoto, K., and Deguchi, Y.: The intestinal microflora of cultured specimens of a puffer *Fugu rubripes rubripes*. Nippon Suisan Gakkaishi., 54, 733 (1988)

11) Kogure, K., Do, H. K., Thuesen, E. V., Nanba, K., Ohwada, K., and Simidu, U.: accumulation of tetrodotoxin in marine sediment. Marine Ecol. Progr. Ser., 45, 303-305 (1988)

12) Kodama, M., Ogata, T., and Sato, S.: Saxitoxin producing bactrium isolatef from *Protogonyaulax tamarensis*. In T. Okaich, D. M. Anderson, and T. Nemoto (eds.). "Red Tide. BNiology, Environmental Science and Toxicology". Elsevier., pp. 363-366 (1989)

13) Moore, R. E., Helfrich, P., and Patterson, G. M. L.: The deadly seaweed of Hana. Oceanus., 25, 54-63 (1982)

14) Kosuge, T., Tsuji, K., Hirai, K., and Fukuyama, T.: First evidence of toxin production by bacteria in a marine organism. Cem. Pharm. Bull., 33, 3059-3061 (1985)